

# SOLID STATE FERMENTATION OF RICE BRAN: NUTRITIONAL VALUES AND FUNCTIONAL PROPERTIES

<sup>1</sup>Shehu Isah, <sup>2</sup>Kennedy Unakalamba

<sup>1</sup>Caleb University, Lagos and <sup>2</sup>University of Abuja, Abuja

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**Abstract:** Solid state fermentation of rice bran improves nutritional values and functional properties. The edible fungus, *Pleurotus Sapidus*, was employed for the solid state fermentation. During fermentation, the sample was withdrawn after ten days and further analyzed. An investigation process was carried out on the solid-state fermented rice bran (RB) in comparison with the unfermented (normal) rice bran. The few analyses that were investigated in comparison with the unfermented rice bran were the density tests (bulk, tapped and compact), water and oil absorption capacities, swelling power, moisture estimate, pH, reducible sugar, and water solubility. From the results, it was found that both the fermented and unfermented rice bran have a pH of 5 and 6, respectively, with absorption capacities of both oil (5% for the fermented sample and 20% for the unfermented sample) and water (10% for the fermented sample and 25% for the unfermented sample), and both samples possess swelling power. This study demonstrated a comparison in the nutritional quality of RB after fermentation with *Pleurotus sapidus* in an attempt to find or improve the functional and nutritional value of rice bran via solid state fermentation. The study therefore proves that the functional and nutritional value of the unfermented sample, which has a higher phenol concentration from spectrophotometry and high ascorbic acid, was better than the fermented sample with lower phenol concentration and less ascorbic acid.

**Keywords:** Solid state fermentation, rice bran, high ascorbic acid.

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## 1. INTRODUCTION

Rice bran oil, containing 90-96% lipid, is considered a healthy food due to its high mono- and polyunsaturated fatty acids content. Within the fatty acid (FA) fraction, palmitic acid (21–26%), linoleic acid (31–33%), and oleic acid (37–42%) are the predominant compounds [1-10]. However, its short shelf life is due to its high lipid percentage and lipase enzymes, which degrade the oil, making it rancid and unsuitable for consumption.

Rice bran and its oil are used for medicinal purposes, particularly in Japan, Asia, and India. They are used for conditions like diabetes, high blood pressure, and cholesterol. Rice bran oil contains substances like Vitamin E, which may help lower cholesterol and reduce kidney stone formation. However, there is limited scientific evidence supporting these claims [12].

### BASIC COMPOSITION OF RICE BRAN

It's apparent that rice bran is a potential source of high-value antioxidants for use as additives in foods, pharmaceuticals, and cosmetics because of its unsaturated fatty acid as synergists for antioxidants [66]: [67]: [68].

Earlier studies showed rice bran compositional distinctiveness such as carbohydrate, protein, fat, moisture, ash, fiber, amylose contents, phytic acid, minerals, vitamin contents, and the Glycemic Index (GI) [69]: [70]. Previous analysis of rice

varieties in a particular region of Bangladesh and found they are composed of protein (7.04%), fat (0.37%), crude fiber (0.26%), and ash (0.58%) in parboiled milled rice [71].

Moreover, aromatic rice varieties showed exciting composition such as moisture (11.25%–15.13%), protein (3.23%–6.21%), fat (0.68%–1.45%), and ash (0.88%–1.46%) [72].

The functional composition of rice bran contains a rich source of bioactive compounds [29]. Rice bran's health benefits and enhanced quality have been reported due to their antioxidant compounds and health benefit. It's apparent that rice bran is a potential source of high-value antioxidants for use as additives in foods, pharmaceuticals, and cosmetics because of its unsaturated fatty acid as synergists for antioxidants [66]; [67]; [68].

A recent study has quantified the functional compounds in rice bran including  $\gamma$ -oryzanol (mixture of lipids derived from rice which occurs mainly in the fat fraction of rice bran and rice bran oil) and vitamin E components (tocopherols) in rice bran oil [73], anthocyanin components in red rice [74], and phenolic acids in various rice varieties [75].

### SUBMERGED LIQUID FERMENTATION (SLF)

Submerged liquid fermentation (SLF) is a method used in industrial manufacturing to produce bio-molecules by submerging enzymes and reactive compounds in liquids like alcohol, oil, or nutrient broth. This process requires liquid media for microorganism growth, but higher water content can decrease concentration and contaminate media. The higher quantity of liquid waste produced leads to dumping issues. SLF is expensive due to the need for processed substrate and large-scale practice, increasing labour costs [13-16].

Solid state fermentation (SFF) is a method used to enhance oil recovery from shredded coconut meat, particularly in Indonesia. It is increasingly being used over submerged liquid fermentation due to its low cost, lack of liquid for media preparation, and lower energy consumption, plant and machinery costs, and labor costs [17]. SSF produces high product yields with higher stability and productivity, but it also has disadvantages like delayed enzyme production and toxic substances. Biotechnological industries face challenges in end product purification, scale-up, and biomass estimation [18]. Rice bran fermentation has been studied for its functional properties, revealing increased nutrient availability, bio-surfactant content, and fatty acid content. This process also allows the release and transformation of phenolic and volatile compounds, further enhancing the potential benefits of fermented substrates [19].

An array of health-promoting value added products have been derived from processed rice bran due to its identified active components such as oryzanols, tocopherols, tocotrienols, phytosterols, nucleotides, dietary fiber content, and phenolic compounds [33]; [34]; [35].

The three main constituent of include; cycloartenylferulateoryzanol A, 24-methlenecycloartanyl ferulateoryzanol C and campesterylferulate, as shown in fig 3, 4 and 5 below.

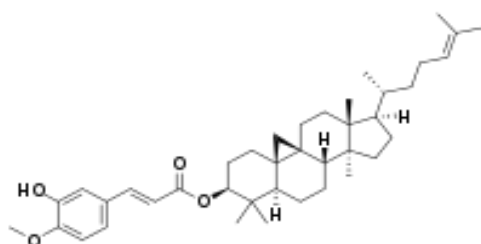


Fig 3: Cycloartenylferulateoryzanol A (C<sub>40</sub>H<sub>58</sub>O<sub>4</sub>)

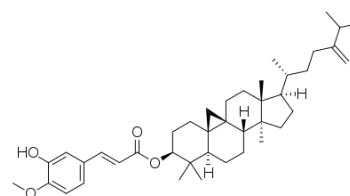


Fig 4: 24-methlenecycloartanyl ferulateoryzanol C

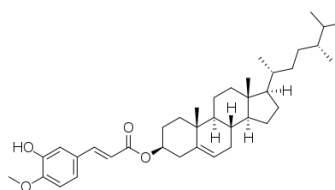
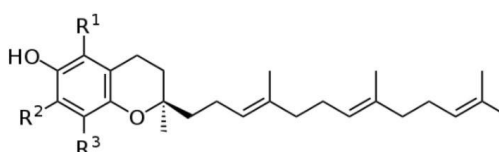


Fig 5: Campesterylferulate (C<sub>38</sub>H<sub>56</sub>O<sub>4</sub>)

As shown in fig. 6 below, both tocopherols and tocotrienols occur in  $\alpha$  (alpha),  $\beta$  (beta),  $\gamma$  (gamma) and  $\delta$  (delta) forms, determined by the number and position of methyl groups on the chromanol ring.

Form	Structure
$\alpha$ -Tocopherol	
$\beta$ -Tocopherol	
$\gamma$ -Tocopherol	
$\delta$ -Tocopherol	

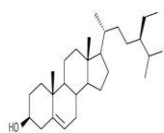
Fig 6: The different forms of tocopherols



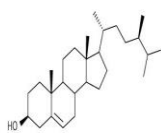
General chemical structure of tocotrienols. *alpha*( $\alpha$ )-Tocotrienol: R1 = Me, R2 = Me, R3 = Me; *beta*( $\beta$ )-Tocotrienol: R1 = Me, R2 = H, R3 = Me; *gamma*( $\gamma$ )-Tocotrienol: R1 = H, R2 = Me, R3 = Me; *delta*( $\delta$ )-Tocotrienol: R1 = H, R2 = H, R3 = Me

Fig 7: Tocotrienol, showing how the different forms can be achieved

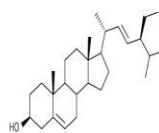
Phytosterols (plant sterol) are a group of naturally occurring compounds found in plant cell membranes, with structure similar to cholesterol. There are two groups; plant sterols (those with double bonds in the sterol rings) and plant stanols (those with no double bonds in the sterol rings)



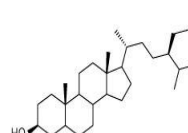
$\beta$ -sitosterol



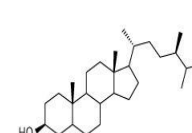
Campesterol



Stigmasterol



Sitostanol



Campestanol

Fig 8: Plant sterols diagram

Fig 9: Plant stanols diagram

Coloured or pigmented rice (purple, black, and red rice) is one of the major food items in an Asian-based diet [38]. The constituents of coloured rice are flavonoids, phenolics, tannin, sterols, tocols,  $\gamma$ -oryzanols, amino acids, fatty acids, phyto-antioxidant compounds, vitamins, and dietary fibers [39]; [40]; [41]. Although rice bran usage is limited due to its rapid rancidity and unfavourable aroma, rice bran fermented with different types of microorganisms makes it an effective agent to retain its potential therapeutic efficacy.

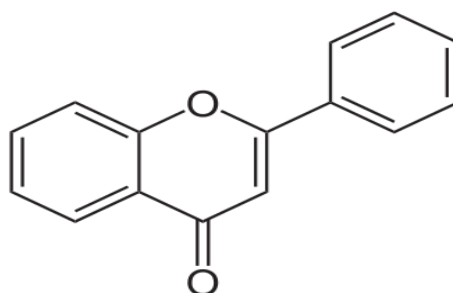
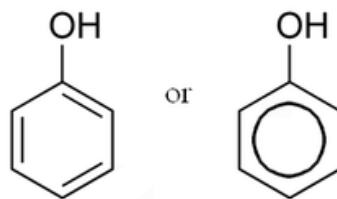
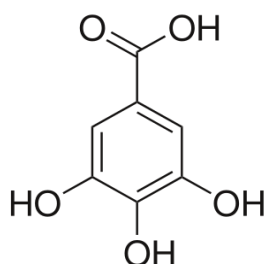


Fig. 11: Flavone, an example of flavonoids

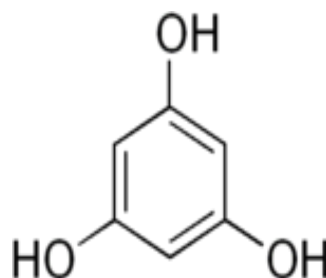


**Fig. 12: Skeletal structure of phenolic compound**

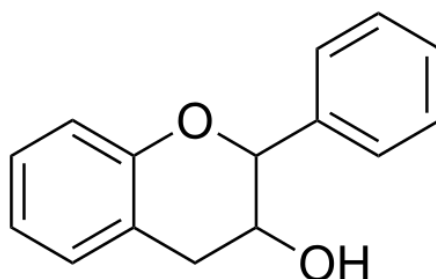
Gallic acid, Phloroglucinol and flavan-3-ol's scaffold are the three base units or monomer of tannin and their classes/polymer are hydrolysable, phlorotannins and condensed tannins and phlobatannins (C-ring isomerized condensed tannins) respectively.



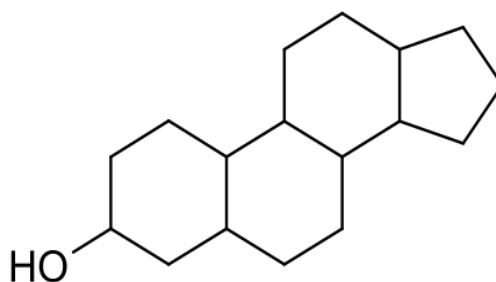
**Fig. 13: Gallic acid**



**Fig. 14: Phloroglucinol**



**Fig. 15: Flava-3-ol's scaffold**



**Fig. 16: Sterols**

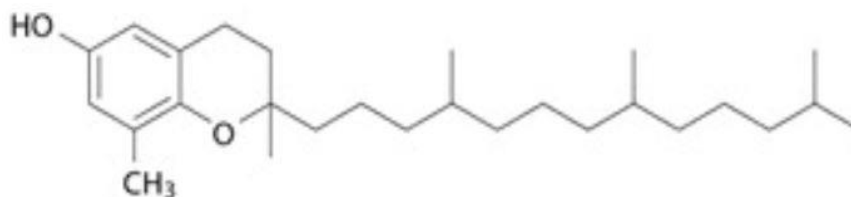


Fig. 17: Tocols

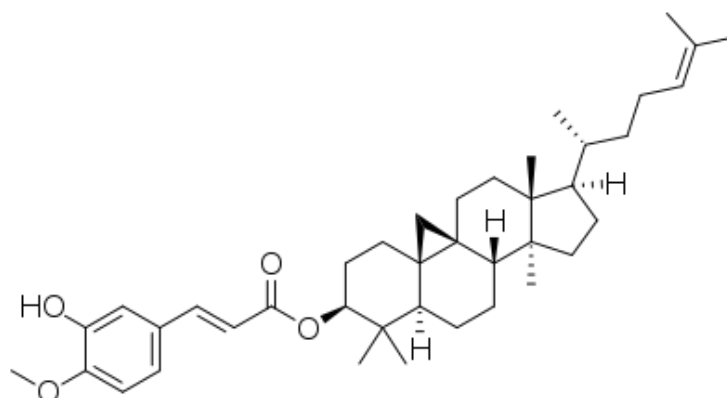


Fig. 18:  $\gamma$ -oryzanol (Cycloartenylferulateoryzanol A)

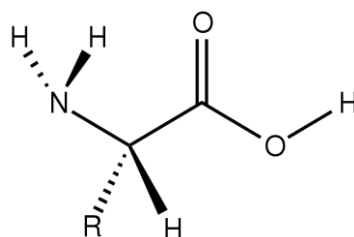


Fig. 19: L-amino acid (an example of amino acid)

The main phyto-antioxidants compounds are polyphenols and carotenoids and their structures are shown below.

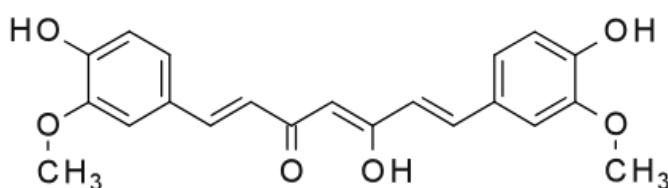


Fig. 21: Curcumin (an example of polyphenol)

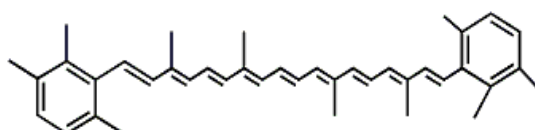
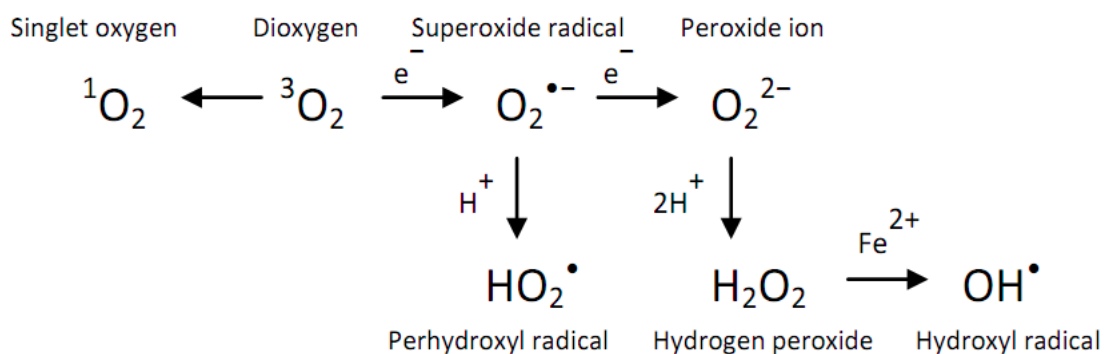


Fig. 22: Isorenieratene (C<sub>40</sub>H<sub>48</sub>) (General structure of carotenoid)

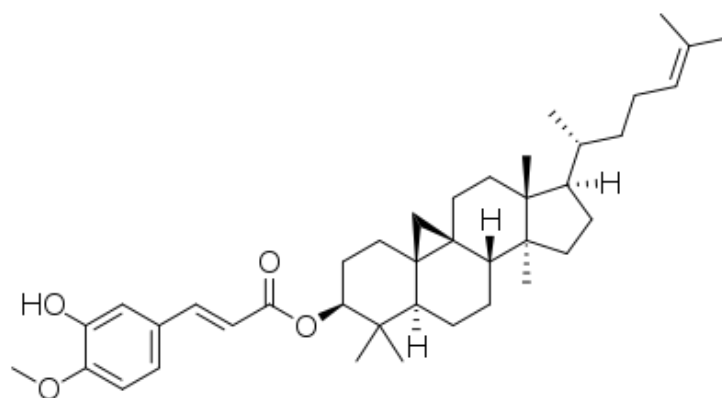
**RICE BRAN EFFECT ON INFLAMMATORY BOWEL DISEASE (IBD)**

Fermented rice bran (FRB) is a bioactive compound from rice bran that has been shown to have protective effects against various diseases, including cancer, metabolic syndrome, obesity, diabetes, and immune modulation. However, there is limited research on FRB's potential against inflammation-related diseases like inflammatory bowel disease (IBD) and multifactorial metabolic disorder (MMD). IBD is a chronic inflammation in the gastrointestinal tract, linked to an increased risk of colon cancer and colorectal cancer. Urbanization, diet, antibiotic use, hygiene, microbial exposures, and pollution are also potential risk factors for IBD and MMD. Injuries in the intestinal mucosa damage the barrier function, leading to inflammation and the production of excessive pro-inflammatory cytokines [42-47].

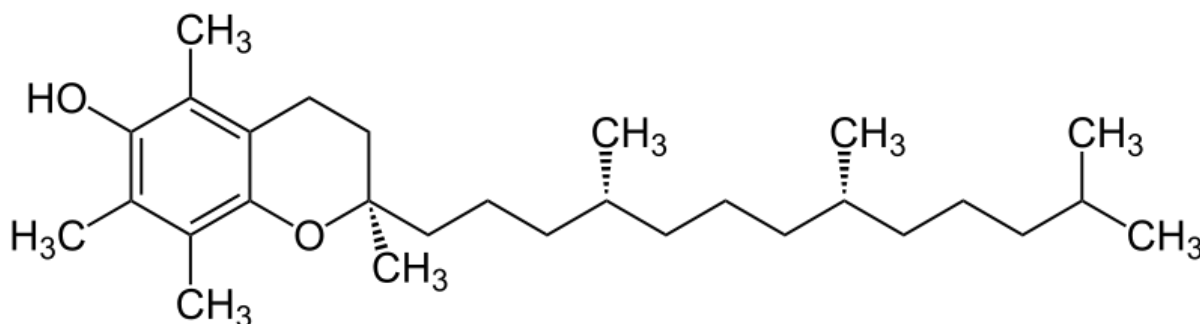


**Fig. 23: Illustration of oxidative stress**

Rice bran has been found to have anti-diabetic and anti-dyslipidemic properties, potentially aiding in treating multifactorial metabolic disorder and colonic disorders. Fermented rice bran with *Saccharomyces cerevisiae* and *Lentinusedodes* has anti-stress, anti-fatigue, anti-cancer, and anti-defective immune responses. Ferulic acid and phenolic compounds have hypoglycemic effects in type 2 diabetic mice, and Driselase-treated rice bran fraction improves glucose and lipid metabolism in SHRSP rats [47,49,51-53].



**Fig. 25:  $\gamma$ -oryzanol**



**Fig. 26: Alpha-Tocopherol (Vitamin E component)**



Fig. 27: Anthocyanin

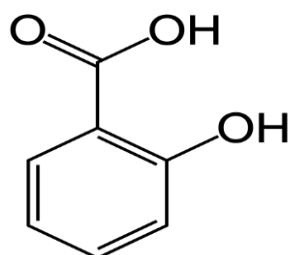


Fig. 28: Salicylic acid (Phenolic acid)

### RICE BRAN-BASED FUNCTIONAL FOOD, A DRUG ALTERNATIVE

Functional food is food products fortified with special constituents with advantageous physiological effects. It can provide health benefits beyond traditional nutrients, as defined by the 1994 National Academy of Sciences' Food and Nutrition Board definition. Functional foods can improve health conditions and homeostatic behavior, using biomarkers or indicators in body homeostasis. Rice bran foods have been found to have a positive effect on human health, with antihypertensive effects.[76-82]

### PREVENTIVE ROLE OF FERMENTED RICE BRAN ON TUMORIGENESIS

The study by Kuno et al (2000) found that fermented brown rice and rice bran with *A. oryzae* (FBRA) can prevent prostate tumorigenesis in transgenic rats. The supplementation decreased adenocarcinoma in the lateral prostate, suppressed prostate carcinogenesis, increased apoptosis, and inhibited cell proliferation in high-grade prostatic intraepithelial neoplasias. It also limited tumor growth by activating energy deprivation pathways, indicating its potential to prevent human prostate cancer [58,83]

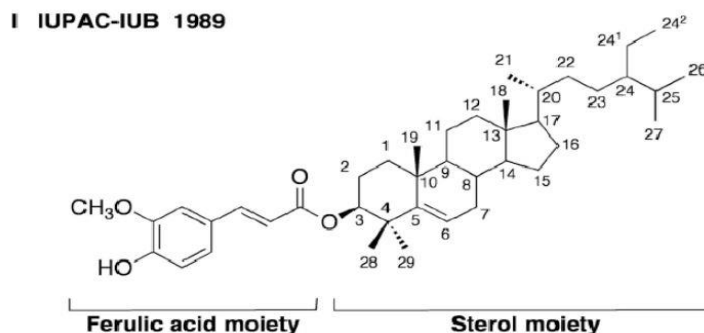
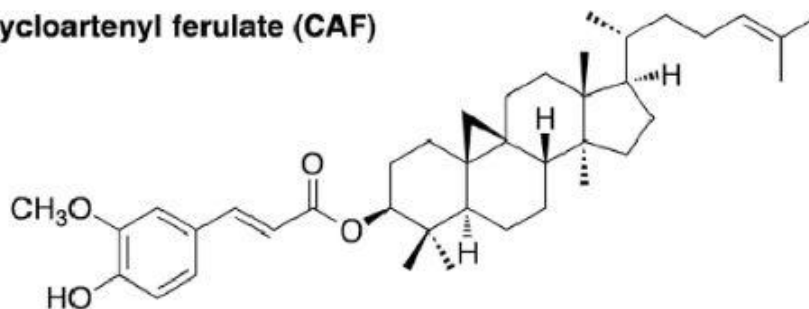
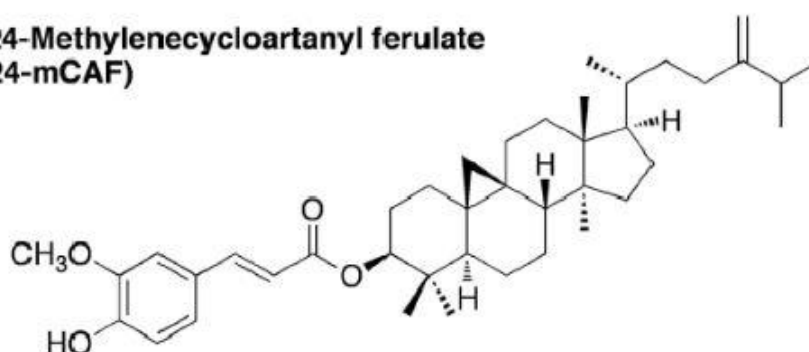
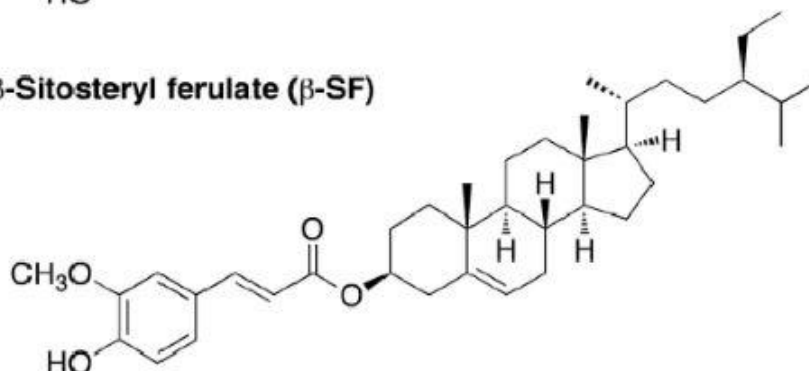


Fig. 29: Chemical structure of phytosterylferulates showing ferulic acid and its sterol moiety

**II Cycloartenyl ferulate (CAF)****III 24-Methylenecycloartanyl ferulate (24-mCAF)****IV  $\beta$ -Sitosteryl ferulate ( $\beta$ -SF)**

**Fig. 30: Some examples of phytosterylferulate: cycloartenylferulate (CAF, principal components of oryzanol), 24-methylenecycloartanyl ferulate (24-mCAF), and sitosterylferulate (SF)**

**SWELLING CAPACITY (SWELLING INDEX)**

The swelling index (SI), also known as swelling capacity (SC), is the volume taken up by one gram of food material under specific conditions. It measures starch's ability to absorb water and swell, reflecting associative forces in granules. Swelling capacity is a quality measure in some food products, such as bakery ones, and is influenced by particle size, species variety, and processing methods[10,100]

**OIL ABSORPTION CAPACITY (OIL ABSORPTION)**

Oil absorption capacity (OAC) is the binding of fat by non-polar side chains of proteins, which enhances mouth feel and retains food products' flavor. It is highly prevalent in foods with high protein content and depends on factors like protein conformation, amino acid composition, and surface polarity. Food substances with good OAC capacities are useful in food applications where optimal oil absorption is desired, such as pastries production. Oil absorption during frying is a concern, as it increases the caloric value of food products. The ideal frying temperature is between 162.78°C (325°F) to 190.56°C (375°F). High OAC food substances are beneficial in structural interactions, improving palatability, extending shelf life, and flavor retention, especially in meat or bakery products. Protein, composed of both hydrophobic and hydrophilic parts, affects OAC capacity[100-103].



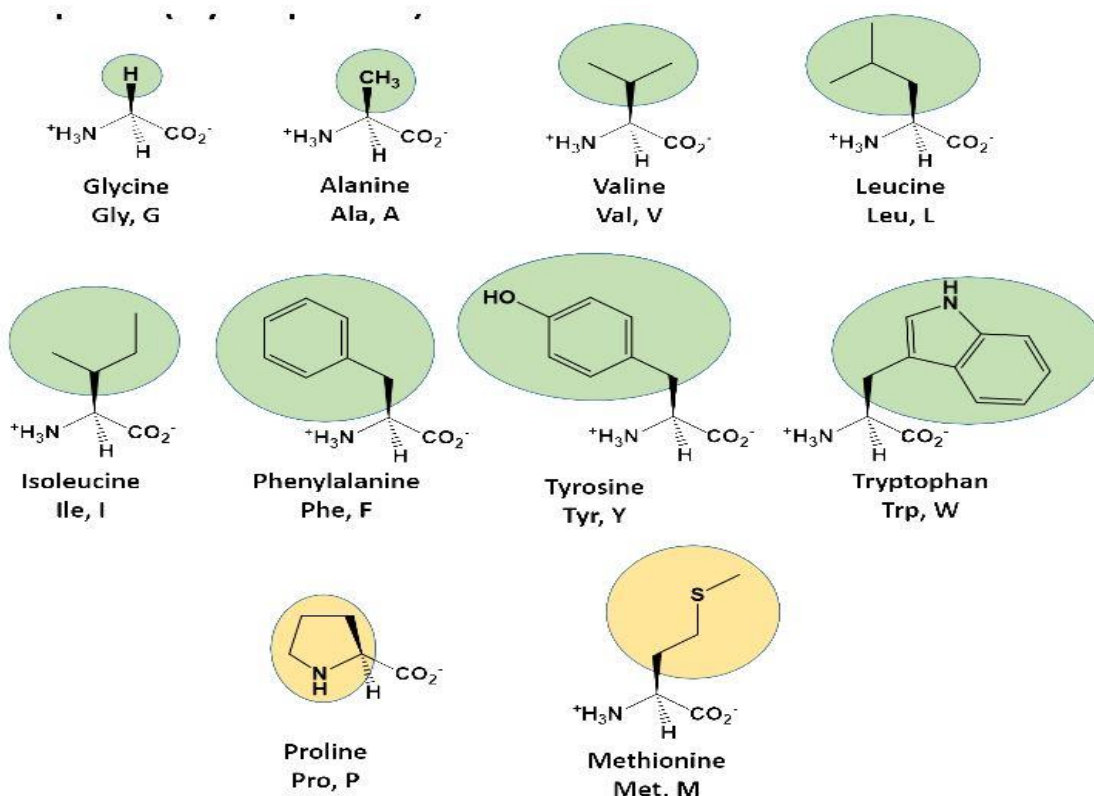


Fig. 31: Binding of oil with protein amino acid

**WATER ABSORPTION CAPACITY (ALSO KNOWN AS WATER HYDRATION OR WATER ABSORPTION)**

Water absorption capacity (WAC) is the amount of water taken up by a substance to achieve desired consistency and quality food products. It is often defined by the weight of the substance. Water absorption occurs when water and food substance mix, hydrating gluten-forming proteins, damaged starch, and other ingredients through hydrophilic interactions and hydrogen bonds with water molecules.

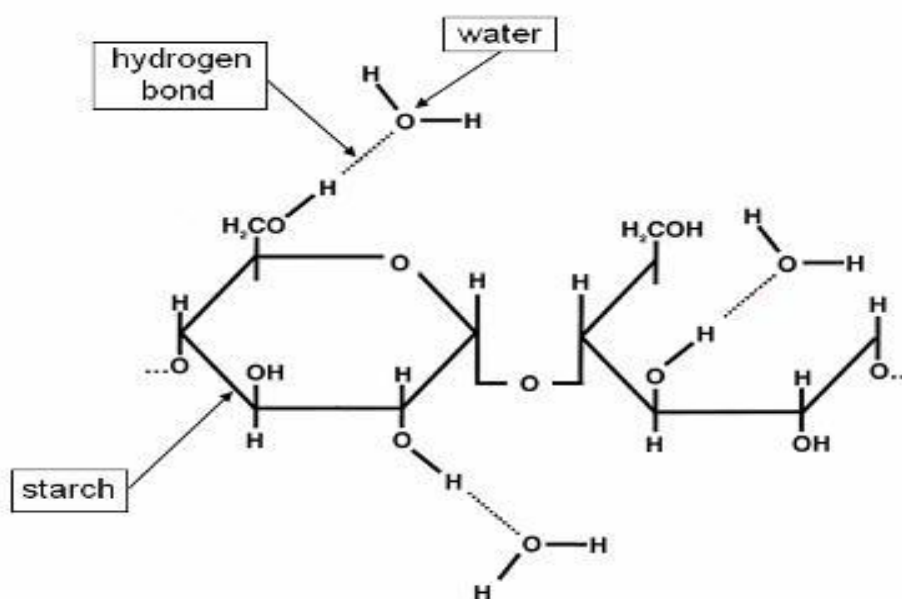
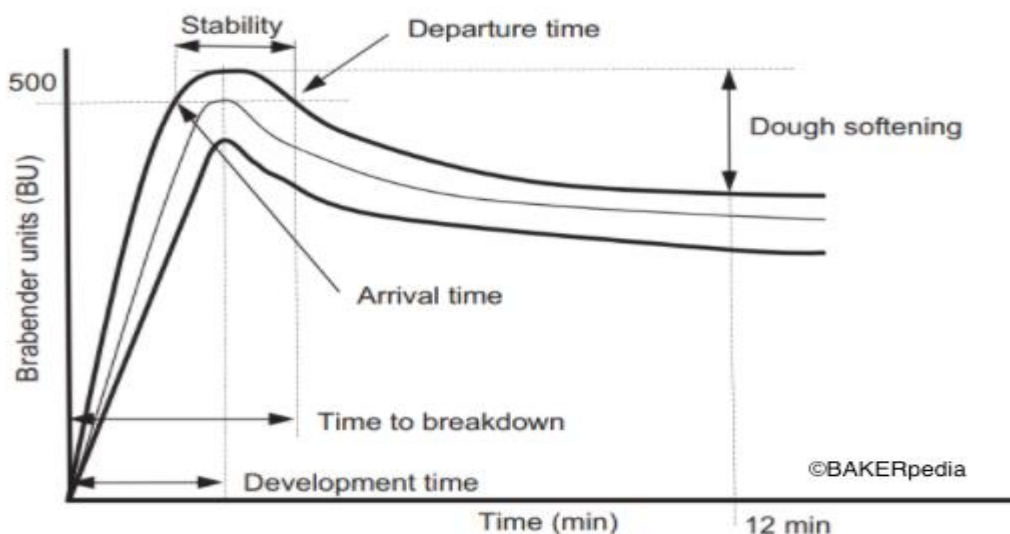


Fig. 32: Hydrogen bonding of water to starch molecules



**Fig. 33: Typical farinograph curve**

Factors influencing the water absorption capacity of a food substance include; starch, protein, pentosans, vital wheat gluten (VWG), and presence of other water binding ingredients such as fiber, bran hydrocolloids (gums).

Water absorption capacity in foods is influenced by hydrophilic components like carbohydrates, proteins, and polar amino acids. Lower absorption in some flours may be due to less availability of polar amino acids. Increased water absorption may also be due to amylose solubility, leaching, and loss of starch structure. High water absorption capacity in composite flours suggests that different flours can be used in food formulations like processed cheese, bakery products, sausage, and dough. Water absorption capacity is crucial for consistency, bulking, and baking applications[100,108-110]

#### **BULK DENSITY (ALSO KNOWN AS VOLUMETRIC DENSITY OR APPARENT DENSITY)**

Bulk density is the mass of many flour particles divided by their total volume. It is a functional property of flours, powders, fine particles, granules, and other divided solids of foods. It can change depending on handling and can be improved by factors such as geometry, measurement method, particle size, surface properties, and solid density. Bulk density also influences the porosity of a food product, which impacts the design of the package and can determine the type of packaging material. Recent studies suggest that flour bulk density may be influenced by the initial moisture content, with high bulk density suggesting suitability for food preparations and low density for complementary foods. Starch forms the main structure and bulk of many food products[101,100].

#### **SOLUBILITY**

Solubility is the ability of food substances to dissolve in a solvent, typically water or oil. It depends on the chemical and physical properties of the solvent and solute, as well as pressure, pH, temperature, and other chemicals. Solubility is measured as the saturation concentration, where more solute does not increase the solution's concentration but precipitates excess solute. Lipids can reduce water absorption capacity, affecting solubility. High solubility indicates high digestibility, making it suitable for infant formula and food. Insolubility refers to the inability of a food to dissolve in a solvent. Water is the most common solvent in food, containing various organic, inorganic, and ionic compounds.[114]

## **2. MATERIALS AND METHODS**

### **STATE FERMENTATION**

10g of Potato Dextrose Agar (PDA) was measured and mixed with 250ml of water. 9ml was measured and kept separately to mix with the sample. The potato dextrose agar solution was then sterilized with the 9ml of water that was measured out separately, in an autoclave machine at 121°C for 15mins. After sterilization, they were allowed to cool for a while and then 10g of the sample (Rice bran) was mixed with the 9ml of sterilized water. The sample solution was poured into the sterilized potato dextrose agar solution and stirred very well. The solution was poured into Petri dishes and labelled, wrapped with

foil paper tightly to avoid reaction with free bacteria in the air, and incubated at 25°C and observed for 10 days. The sample that was gotten after 10 days was then used for further analysis in comparison with the normal, dry unfermented sample.



**Fig. 51: Fermented sample (After 10 days)**

#### **BULK DENSITY ( $p_B$ )**

3g of sample, fermented and unfermented, was measured separately and poured into a measuring cylinder, and then the volume was observed and recorded. The mass of the sample (3g) was divided by the volume, which gave the bulk density.

#### **TAPPED DENSITY ( $p_T$ )**

3g of sample, fermented and unfermented, was measured separately and poured into a measuring cylinder, “tapped severally”(in this experiment, it was tapped 6 times), and then the volume was observed and recorded. The mass of the sample (3g) was divided by the tapped volume, which gave the tapped density.

#### **COMPACT DENSITY**

5g of sample, fermented and unfermented, was measured separately and poured into a measuring cylinder, “compressed” (like pounded) until no further reduction in volume, and then the volume was observed and recorded. The mass of the sample (5g) was divided by the compressed volume, which gave the compact density.

#### **CARR INDEX ( $C$ )**

$$C = 100 \left( 1 - \frac{p_B}{p_T} \right)$$

Where  $C$  = Carr index,  $p_B$  = Bulk density and  $p_T$  = Tapped density

The result from the tapped density ( $p_T$ ) and bulk density ( $p_B$ ) was used to calculate the Carr index, using the formula above.

#### **HAUSNER'S RATIO ( $H$ )**

$$H = p_T/p_B$$

The result from the tapped density ( $p_T$ ) and bulk density ( $p_B$ ) was also used to calculate the Hausner's ratio, using the formula above.

#### **WATER ABSORPTION CAPACITY**

10ml of distilled water was mixed with 1g of sample (both samples, separately) and allowed to settle for 30mins after which it was centrifuged at 2000rpm for 10mins. After centrifuge, the liquid was separated from the solid and the weight and volume was observed and recorded.

### OIL ABSORPTION CAPACITY

10ml of groundnut oil was mixed with 1g of sample (both samples, separately) and allowed to settle for 30mins after which it was centrifuged at 2000rpm for 10mins. After centrifuge, the liquid was separated from the solid and the weight and volume was observed and recorded.

### SWELLING POWER

14ml of distilled water was mixed with 0.3g of sample (both samples, separately) and heated up to 73°C and allowed to cool at room temperature for a while. After cooling, it was centrifuged at 5000rpm for 10mins. The fermented and unfermented sample was separated from the water after centrifuge, and the weight of both samples were recorded.

### ESTIMATION OF MOISTURE

Estimated moisture = (lost weight / initial weight) × 100

3g of both samples were measured separately and put in petri dishes and then put in an oven to dry at 105°C. A check was done at interval of 30mins until constant weight was obtained.

### PH TEST

1g of both samples was mixed with 10ml of distilled water separately and then tested with litmus paper and the visible colour change of the litmus paper was observed.

### REDUCIBLE SUGAR

1g of sample was mixed with 10ml of distilled water separately, and then 5 drops of Benedict's reagent (sodium carbonate, sodium citrate and copper (II) sulphate pentahydrate) was added to both separate mixture of samples and then heated up to 75°C and observed for any colour change which will indicate the presence of reducible sugar.

### WATER SOLUBILITY

0.3g of both samples were added to 0.5ml of distilled water and then tapped to observe if any particle goes down. A solution of the sample was also prepared separately, and then added in water in drops and observed.

### SPECTROPHOTOMETRIC METHOD FOR PHENOLIC COMPOUND ( $\gamma$ -ORYZANOL) DETERMINATION

A mixture of 4ml of phenol was added to 1g of fermented and unfermented samples, mixed with ferric chloride, distilled water, and distilled water. The mixture was then diluted with distilled water and tested using a UV spectrophotometric machine. The concentration of phenol was determined using Beer Lambert's principle of absorbance, which was then treated with the molar attenuation coefficient of  $\gamma$ -oryzanol, a phenolic compound in rice bran.

### ASCORBIC ACID

A little light blue solution of dichlorophenolindophenol (DCPIP), already diluted, was put in a 2 beakers, separately, for the 2 samples. Both samples solutions (fermented and unfermented) were prepared also. With the use of a pipette, a clear solution of the samples was drawn and then added to the dichlorophenolindophenol solution in the beaker, 1 drop at a time, to observe for colour change (blue to red or pink), which signifies the presence of ascorbic acid.

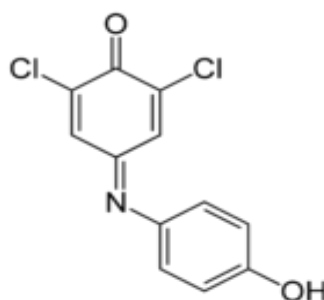


Fig. 52: (DCPIP) 2,6-dichlorophenolindophenol (C<sub>12</sub>H<sub>7</sub>Cl<sub>2</sub>NO<sub>2</sub>)

### 3. RESULTS AND DISCUSSION

From the analysis carried out, table 1 below is the result, showing the bulk density, tapped density, compact density, Carr index and Hausner's ratio of the fermented and unfermented sample.

**Table 1: Density tests, Carr index and Hausner's ratio**

TESTS	FERMENTED SAMPLE	UNFERMENTED SAMPLE
Bulk Density	0.3g/ml	0.286g/ml
Tapped Density	0.75g/ml	0.3g/ml
Compact Density	0.833g/ml	0.385g/ml
Carr Index	60	4.67
Hausner's Ratio	2.5	1.05

#### BULK, TAPPED, AND COMPACTED DENSITY

These are functional properties of powdery substances or particles which can be used to determine the starch content in food particles and the higher the starch content, the more likely the increase in these densities. More also when there is an increase in the volume, then there will be a decrease in the bulk, tapped and compacted density, which indicates lower starch content. High bulk density also suggests suitability for application in food preparation. On the other hand, low density can also be useful in the formulation of complementary foods.

So from the result above, the fermented sample obviously has a higher bulk, tapped and compacted density therefore contains more starch content. In other words, fermented sample is more suitable for application of food preparation. Meanwhile, on the other hand, the unfermented sample with low density would be more useful in the formation of complementary foods.

#### CARR INDEX

Carr index is an indication of the compressibility of a powder and is frequently used in pharmaceuticals. In a free-flowing powder, the bulk density and tapped density would be close in value; therefore, the Carr index would be small. On the other hand, in a poor-flowing powder where there are greater inter-particle interactions, the difference between the bulk and tapped density observed would be greater, therefore, the Carr index would be larger<sup>[147]</sup>. A Carr index greater than 25 is considered to be an indication of poor flowability, and below 15, of good flowability<sup>[148]</sup>.

The bulk and tapped density of the fermented sample is not close, from the table above, and obviously, the carr index is very large (greater than 25), therefore it is poor-flowing and has greater inter-particle interaction. On the other hand, the bulk and tapped density of the unfermented sample is very close, and the Carr index from the table is very low (below 15), so it is a good-flowing powder.

#### HAUSNER'S RATIO

Hausner's ratio is an indication of the flowability of a powder<sup>[155]</sup>, and it's much related to Carr index. A Hausner ratio greater than 1.25 - 1.4<sup>[156]</sup> is considered to be an indication of poor flowability.

The table above shows the Hausner's ratio of both the fermented and unfermented sample, after due calculation. The fermented sample can be considered to have poor flowability because it has a ratio above 1.25 - 1.4.

#### Other tests;

Table 2 below, shows the final results for the water/oil absorption capacity, swelling power, estimated moisture content, pH and the phenolic compound determination via spectrophotometry for both the fermented and unfermented sample.

**Table 2: Water/oil absorption capacity, swelling power, estimation of moisture, pH and spectrophotometric determination of phenolic compound (*γ-oryzanol*)**

TEST	FERMENTED SAMPLE	UNFERMENTED SAMPLE
Water Absorption Capacity	10%	25%
Oil Absorption Capacity	5%	20%
Swelling Power	0.1g	0.8g



Estimation of Moisture	97%	12.3%
Ph	5	6
Spectrophotometric Determination of Phenolic Compound ( $\gamma$ -Oryzanol)	0.0105m/cm	2.105m/cm

**ESTIMATION OF MOISTURE**

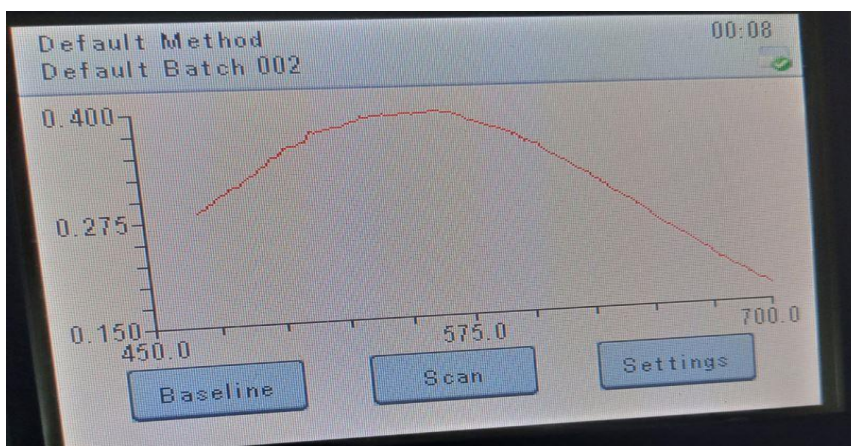
The table below (Table 3) shows the different weights of 3g of both samples each, in the oven at interval of 30mins check.

**Table 3: Different weights at 30mins interval**

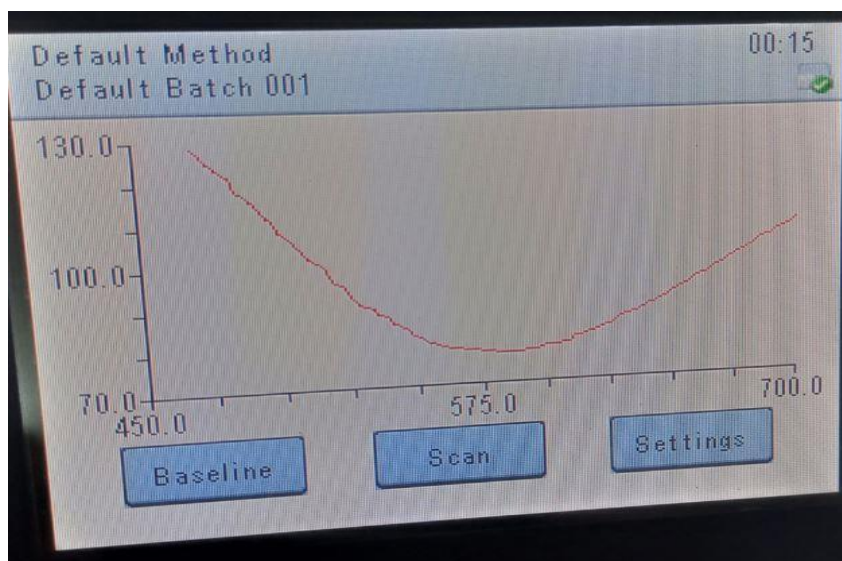
	Initial weight	After 30mins	After 1hr	After 1:30mins	After 2hrs	After 2:30mins	Weight Lost
<b>Fermented Sample</b>	3g	1.56g	0.18g	0.11g	0.09g	0.09g	2.91g
<b>Unfermented Sample</b>	3g	2.64g	2.63g	2.63g	2.63g	2.63g	0.37g

**SPECTROPHOTOMETRIC DETERMINATION OF PHENOLIC COMPOUND ( $\gamma$ -ORYZANOL)**

Spectrophotometric determination involves wavelength and infrared rays passing through a photometric machine to determine  $\gamma$ -oryzanol concentration. This knowledge is crucial in pharmaceuticals, especially for determining medication dosage.  $\gamma$ -oryzanol is used in Japan for menopausal symptoms, anxiety, stomach upset, and high cholesterol. Fermented samples have lower concentrations, while unfermented samples are richer.[158]



**Fig. 58: Spectrophotometric phenol test for fermented sample**



**Fig. 59: Spectrophotometric phenol test for unfermented sample**

The result for reducible sugar, water solubility and ascorbic acid test for both the fermented and unfermented samples are shown below.

**Table 4: Reducible sugar, water solubility and ascorbic acid**

TEST	FERMENTED SAMPLE	UNFERMENTED SAMPLE
Reducible Sugar	No colour change observed	No colour change observed
Water Solubility	No reaction at all, it did not dissolve.	No reaction at all, it did not dissolve. Kept going down after each tap and when stirred, it mixed, but after settling for a minute, there were particles below. Therefore it's not totally soluble
Ascorbic Acid	A very little colour change from blue to pink was observed	From initial light blue to pink colour change was observed

**REDUCIBLE SUGAR**

Benedict’s reagent was added to both separate mixture of samples and then heated up to 75°C and observed for any colour change which will indicate the presence of reducible sugar. In this case, both the fermented and unfermented samples had no colour change, which signifies that there is no reducible sugar present for both sample.

**WATER SOLUBILITY**

Food substance solubility is the amount of the food substance that dissolves into solution, usually with water as solvent. The presence of lipids, which reduces the water absorption capacity of food, also leads to reduced solubility [113]. High solubility of food can show high digestibility of the food which may indicate excellent use for infant formula and food.

From the table above, the fermented sample had no reaction at all, meaning it was completely insoluble, but the unfermented sample can be said to be a bit soluble (not completely soluble). Both samples can be said to possess lipids which makes them insoluble or not completely soluble, making them low digestible and poor use for infant formula and food.

**ASCORBIC ACIDS**

Ascorbic acid also known as vitamin C is a vitamin found in various food substances and can be used for treatment of scurvy (a deficiency of vitamin C) [129]. It also functions as an antioxidant.

The two samples’ solution (fermented and unfermented) were added to the light blue solution of diluted dichlorophenolindopheol (DCPIP) one drop at a time to observe for colour change (blue to red or pink), both had colour change.

For the fermented sample, there was a delay in the colour change; a reasonable amount had to be poured before a slight colour change was observed as shown in fig. 61 below, which signifies presence of little ascorbic acid. A few drops of the unfermented sample, changed from blue to pink.

**4. CONCLUSION**

Rice bran is a promising nutritional and functional food that more attention should be paid to for more research and analysis. More comparison with other food substances should be done.

Fermented rice bran appears to be another part that should be experimented on as well because this research only focused on few analyses in comparison with the unfermented (normal) rice bran.

Based on this research, it can be concluded that from the comparison of result, the unfermented rice bran has more potentials when it comes to food production and pharmaceuticals than the fermented rice bran, apart from the starch content. Further analysis can be investigated to prove otherwise, but from the few done on this research, this is just the conclusion.

Unfermented rice bran possesses more protein (especially amino acids), more carbohydrate (especially polysaccharides), which makes it more palatable, better shelf life and good flavour retention than the fermented sample. In other words, the

nutritional value of the unfermented sample can be said to be better than the fermented sample. In as much as the fermented sample possesses more starch than the unfermented sample, the starch in the unfermented has the ability to absorb water better than the fermented sample that is more of moisture, which makes it more palatable in nutrition. The moisture in the fermented sample also makes it unsuitable for food production and reduces its shelf life, giving room for microbial growth.

Functionally, it can also be said that the unfermented sample is of more value than the fermented sample because of its high flowability, high ascorbic acid and high phenol concentration which can be used in pharmaceuticals. Cholesterol control, multifactorial metabolic disorder (MMD), and inflammatory bowel disease (IBD) can be taken care of with rice bran (unfermented). Tumorigenesis on the other hand can be prevented by fermented rice bran <sup>[83]</sup>.

So as earlier stated, unfermented rice bran possess more nutritional and functional value than the fermented rice bran from the few analysis carried out in this research. More research should be done on rice bran as it appears to be a promising functional food.

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